Report for 2001ME2221B: Do Microorganisms Control Arsenic Mobility in Groundwater?

- Conference Proceedings:
  - 1. McCaffery, K., and MacRae, J.D. 2002. Microbial arsenate reduction in anaerobic groundwater. Poster presentation at the Maine Water Conference, May, 2002.
  - 2. McCaffery, K., and MacRae, J.D. 2002. Microbial arsenate reduction in anaerobic groundwater. Poster presentation at Arsenic in New England: a Multidisciplinary Scientific Conference, May, 2002

Report Follows:

Progress Report 2002.

Role of microorganisms in arsenic contamination of groundwater.

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Publications:

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Abstract:

Arsenic contamination has emerged as a problem in groundwater drinking water supplies in Maine. Since arsenic exposure through drinking water has been linked to increased risk of cancer, this issue is of grave public health concern. Arsenic speciation affects its adsorption and mobility. The reduced inorganic form, As(III), is more mobile and more toxic than the more oxidized form, As(V).

Microorganisms can affect the redox chemistry of arsenic compounds. Under reducing conditions, which are usually encountered in groundwater, microorganisms can catalyze the reduction of As(V) to As(III) in energy-generating reactions. Other microorganisms cause the release of adsorbed arsenic through reduction and dissolution of Fe(III) and Mn(IV). These transformations result in an increase in soluble arsenic and could contribute to contamination of the groundwater.

Information on the magnitude of these microbial processes in the groundwater environment and their role(s) in As release and solubility is needed to improve As management options for water supplies.

Enrichment cultures of microorganisms that can reduce As(V) have been made from groundwater and the scrapings from a well casing. Stable cultures of arsenate-reducing bacteria have been obtained. Characterization of these cultures is ongoing. Solid and liquid samples will also be taken from contaminated wells and experiments will be conducted to characterize Fe(III), Mn(IV) and As(V) reduction and release of As from the geologic matrix. The effects of organic carbon enrichment (lactate addition) on the reduction reactions and dissolution of arsenic will also be investigated to simulate the effect of increased organic loading on water quality.

Problem

Arsenic has been recognized as a potent human toxin for well over a century. Chronic exposure to lower concentrations through drinking water also causes cancer and other adverse health effects. Approximately 10% of wells sampled in the United States by the U.S.G.S. contained >10 µg/L As and much of Maine is mapped as having anomalously high arsenic in groundwater. Several regions of Maine contain wells that have arsenic concentrations in the ppm range. These elevated arsenic concentrations and "hotspots" such as the towns of Northport, Buxton, and Ellsworth pose an unacceptable health risk to the exposed populations.

It is widely reported that arsenic solubility increases with decreasing redox potential. Microbial activity in the presence of organic material consumes oxygen and other oxidants, producing conditions that favor arsenic mobility. A definition of the processes that affect arsenic form and mobility will result in better management options for arsenic control. The underlying risk factors need to be established for Maine so that preventive, rather than the more expensive end-of-pipe or point-of-use control options can be developed and employed.

There are a number of ways in which arsenic may be mobilized by microorganisms, but the direct reduction of arsenic has not yet been demonstrated in groundwater. The purpose of this research is to identify the ways in which microorganisms participate in arsenic transformations and mobility in groundwater.

Research Objectives

- To obtain bacterial enrichments or isolates of arsenic reducers from arsenic-contaminated wells for further study on mechanisms and controlling parameters.
- To characterize microbial reduction of Fe(III), Mn(IV) and As(V) in water and sediment slurries made from samples taken from arsenic contaminated wells.
- To evaluate the effect of addition of an easily utilizable carbon source (lactate) on Fe(III), Mn(IV) and As(V) reduction rates.

Methods

Sample Collection and Analysis. Water samples were collected by pumping water into nitrogen-purged serum bottles to maintain sample redox potential. Solid material from a well casing was scraped off with a sterile spatula and added to reduced media under a stream of nitrogen. Arsenic was speciated in the field by ion chromatography. Other water quality parameters were measured using standard methods. Arsenic speciation in enrichments was determined by HPLC or ion-exchange chromatography and ICP analysis. For monitoring of enrichment cultures, Fe(II) will be analyzed by the oxalate extraction-ferrozine method.

Microbiological Analysis. Laboratory microcosms will be prepared in 125 ml serum bottles using ground bedrock from a well in Northport and groundwater (1:4 slurry). Sterile controls will also be included to account for abiotic transformations. Reduction of added iron, manganese and arsenate will be monitored over time by analysis of reduced electron acceptors in the supernatant. Sulfide will also be measured, and all species determined in both solid and liquid phases at time 0 and at the end of the experiment. Incubations will be in the dark at 22 °C. Microcosms (live and killed) will also be prepared with 5 mM lactate added (without added electron acceptors) to determine if the rates of reduction and arsenic release are affected by organic amendment.

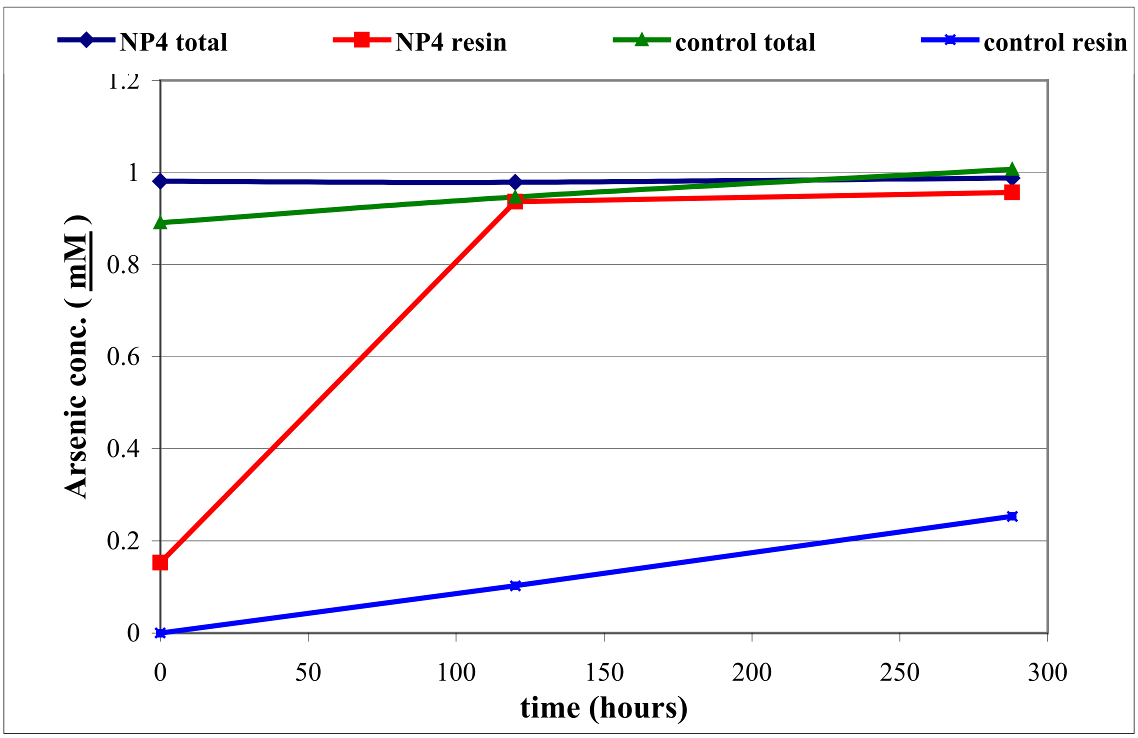
Arsenic-reducing enrichments were made by adding scrapings from the well casing to minimal media containing 5 mM sodium arsenate and 5 mM lactate as the carbon/electron source using strict anaerobic techniques. Alternatively, groundwater was supplemented with nutrients and incubated anaerobically. Stable enrichments were monitored for growth and loss of substrates and changes in electron acceptors over time. 16S rDNA from isolates will be amplified from DNA extracts by the polymerase chain reaction and sequenced for tentative identification of the strains.

Principal Findings to Date

Early analysis of enrichment media showed that at least one culture from each site (Northport and Green Lake) could reduce As(V). A stable enrichment culture that could use arsenic (V) as an electron acceptor was obtained from the Northport groundwater samples. The use of lactate as electron donor and As(V) as electron acceptor was confirmed by HPLC analysis of the culture media over time. The growth modes of other cultures have not yet been confirmed by analysis of the media, although growth occurred and cells were observed by microscopy.

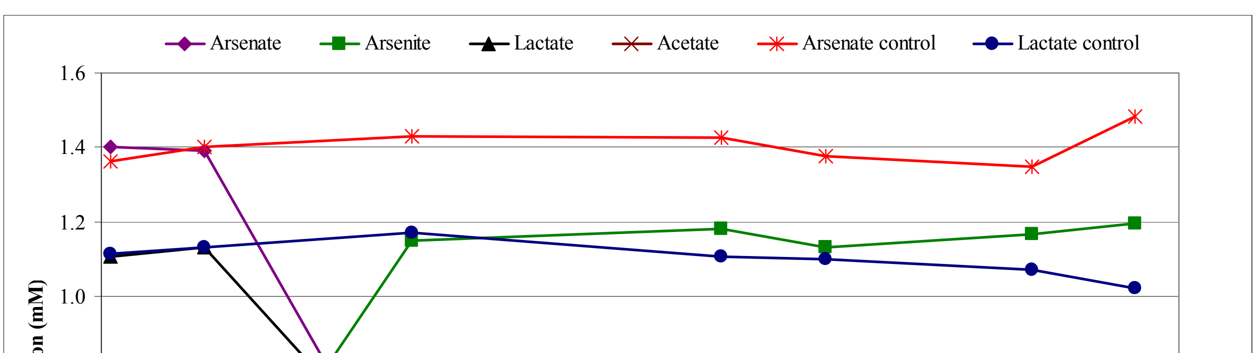
An early experiment was conducted using an enrichment culture from Northport showed that 1 mM As(V) was converted to As(III) in approximately 5 days (Figure 1).

Figure 1. As(III) and total As in NP4 culture medium with time



Another experiment was conducted using the HPLC method to analyze the culture medium for lactate, acetate, As(III) and As(V). Killed controls were also included. Figure 2 shows that as lactate and As(V) were consumed, As(III) was produced to a maximum of 90% of the total As concentration. Acetate was also produced but not with a 1:1 stoichiometric relationship. Presumably this is due to the production of biomass. The lactate and arsenate levels in the controls did not significantly change with time.

Figure 2: Growth of NP4



The enrichment is being tested to determine if it is an isolate by PCR analysis and sequencing. This will also allow us to determine the identity of the organism(s). Experiments are also underway to determine the profiles of carbon source and electron acceptor usage.

A second graduate student is beginning the incubations with bedrock material and groundwater this summer. These laboratory analyses will allow us to determine the conditions under which arsenic is released from the solid matrix.

Funding has been awarded by NSF to continue the analysis of the enrichment culture/isolate and to develop a genetic probe method to assess the relative importance of this organism in environmental samples. Additional enrichments and incubations will be made to determine the importance of indirect modes of action upon As mobility such as iron and manganese reduction. Additional funding will be sought from EPA to assess the importance of organic enrichment on As mobility, such as near landfill sites, intensive livestock rearing and composting facilities.

Microbial Arsenate Reduction in Anaerobic Groundwater

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We present findings that indicate dissimilatory arsenate reduction is occurring in anaerobic groundwaters of Maine. Reduction of arsenate, accompanied by oxidation of an organic carbon compound (lactate) has been observed in lab cultures obtained from contaminated groundwater samples.